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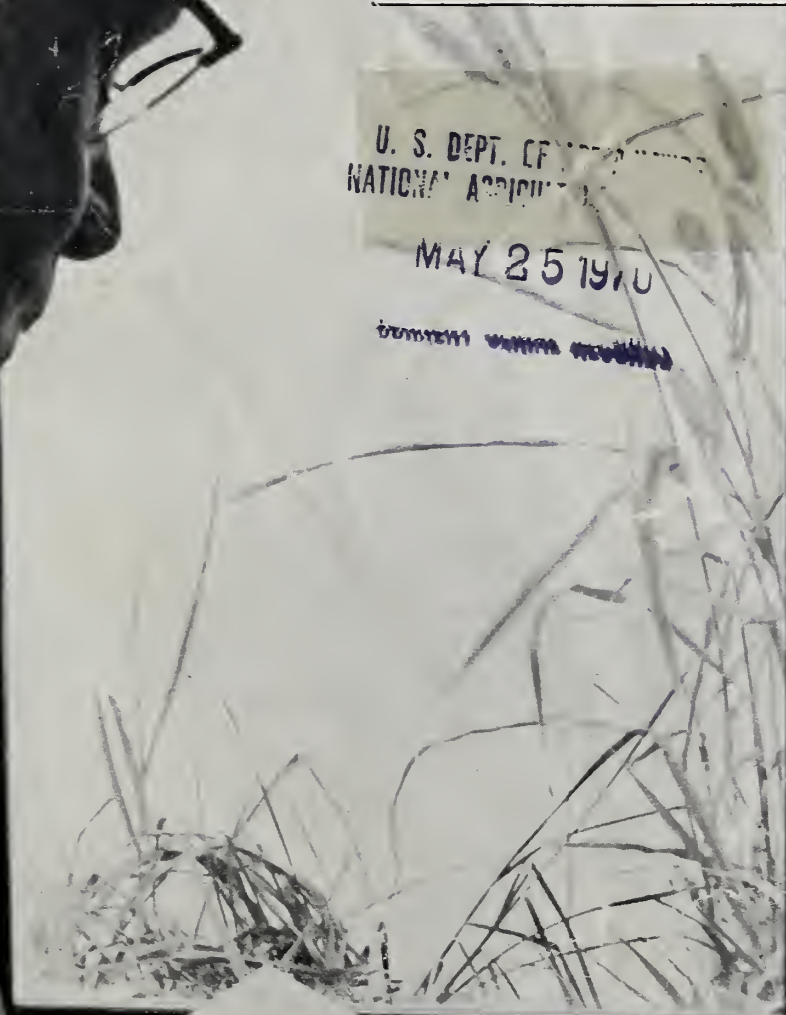
# AGRICULTURAL Research

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# AGRICULTURAL Research

May 1970/Vol. 18, No. 11

## A Vital Link

Every spring thousands of visitors converge on the National Arboretum to savor a hillside aflame with the bloom of 70,000 azaleas. The azaleas are justly famed. But relatively few visitors know about other noteworthy plantings—the Fern Valley Trail, for example, whose plantlife evokes the visual charm of a lush slope in Appalachia or the somber beauty of a woodland bog.

The display plantings are but one of many programs at the Arboretum, a 415-acre tract in Washington, D.C., set aside by Congress in 1927 to advance “research and education concerning trees and plantlife.” Its Herbarium, for example, houses a collection of some 350,000 dried and carefully preserved specimens. With a major acquisition dating from 1820, the collection is globally known for the wealth of its cultivated and ornamental plant species, many of them not available elsewhere. It provides an invaluable reference and identification service for scientists, plantmen, and home gardeners.

A long-term mission of the Arboretum, which is a part of ARS, is to develop new decorative woody plants that are pest-resistant and hardy for American landscapes. The scientists work with breeding material from a gene bank containing promising native species as well as exotics collected by plant explorers. Their task is time consuming. To develop a new shrub may take 10 years; a new shade tree, 20 years. In recent times ARS scientists have been manipulating day length and temperature to cut years from flowering time, thus speeding the availability of progeny for selection and testing.

Better shade trees are urgently needed to enhance the living and working environment of our increasingly urbanized society. This quest is difficult, for today's cities and towns are becoming increasingly inhospitable for trees. Stresses include not only insects and diseases but also polluted air, droughty soils, road salt, and occasionally soil compaction.

The world of growing things helps sustain the human spirit. Although man throngs the cities he still moves to ancient rhythms. The weekly exodus to the country basically reflects a biological urge to maintain contact with the kind of environment from which we evolved. For man is a part of nature. Trees, shrubs—indeed all growing things—provide a vital link with our primeval heritage.

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**Clifford M. Hardin, Secretary**  
**U.S. Department of Agriculture**

**G. W. Irving, Jr., Administrator**  
**Agricultural Research Service**





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*Isolated at last—*

## the boll weevil's grandlure

*Attractants to lure insects into traps or otherwise aid in control are receiving widespread attention because they offer the promise of a substitute, or supplement, to pesticides. In the search for effective boll weevil attractants, the step-by-step research achievements read like a continued story. Last year in January, for instance, AGRICULTURAL RESEARCH reported that boll weevils were drawn to cotton more by male weevils than by any attractant the plant itself may have. In April, 1969, we reported that sticky traps baited with live male weevils looked promising and in October, the success of a field trial utilizing such traps. In the latest chapter, ARS scientists have isolated the attractant itself.*

THE BOLL WEEVIL may be synthesizing its attractant from the plants it feeds upon. ARS scientists have isolated four compounds from boll weevils which, when mixed together, produce an active synthetic attractant they named grandlure.

Pinning down this attractant was an extremely complex problem because of the combination of four compounds—all having unusual chemical structures. Most insect attractants contain only one. The scientists, stationed at the ARS Boll Weevil Research Laboratory, State College, Miss., theorize that the weevil is forming these compounds from a plant constituent because terpene alcohols in cotton plants can be converted to the compounds.

In the boll weevil species, the lure is manufactured by the male not only to attract females, but also, seasonally, to attract other males to feeding sites. Earlier studies by ARS entomologist D. D. Hardee indicated that production of the attractant appears to be related to the weevils' diet, as males attract other weevils only after feeding. Moreover, males feeding on fresh cotton buds or cotyledons are more attractive to females than are males



feeding on an artificial diet used in laboratory rearing.

In laboratory tests, females responded to grandlure in the same way that they reacted to live male weevils. However, the artificial sex attractant, unit per unit, is probably not equal in potency to the natural lure of the males, but more of the artificial attractant can be applied in traps than can be produced by the males usually caged in traps.

A team of six scientists—chemists J. H. Tumlinson, R. C. Gueldner, A. C. Thompson, J. P. Minyard, Jr., and P. A. Hedin, along with Hardee—isolated the lure from extracts of 4.5 million weevils of both sexes. In addition, the attractant was found in the males' feces.

The extracts consisted of two alcohols: *cis*-2-isopropenyl-1-methyl-cyclobutane-ethanol and *cis*-3,3-dimethyl- $\Delta^1,\beta$ -cyclohexaneethanol, and two aldehydes: *cis*-3,3-dimethyl- $\Delta^1\alpha$ -cy-

clohexaneacetaldehyde and *trans*-3,3-dimethyl- $\Delta^1\alpha$ -cyclohexaneacetaldehyde. Absence of either alcohol or both aldehydes from the mixture resulted in almost complete lack of response by the females.

The scientists obtained the best laboratory response from the females by using a mixture containing 0.99 microgram ( $\mu\text{g}$ ) of the first alcohol, 0.07  $\mu\text{g}$  of the second alcohol, and 0.12  $\mu\text{g}$  each of the two aldehydes.

In laboratory tests, grandlure attracted only females. Field tests in the spring and fall produced responses by both sexes, but in midsummer, the lure attracted mostly females. These seasonal variations occurred with both natural and synthetic lures.

Further tests are planned to determine the potential for grandlure in survey traps and possibly in control of boll weevils. The Mississippi Agricultural Experiment Station, State College, is cooperating in this project. ■



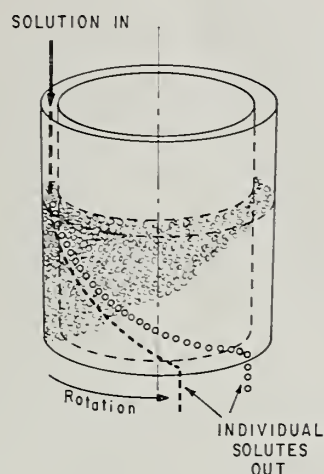
*Above: Pellet containing synthetic attractant is put into cage that normally housed male weevils used as lures. Cages are placed in traps covered with sticky coating (PN-1867).*

*Below: The team that isolated the attractant—Tumlinson, Hardee, Minyard, Hedin, and Gueldner. Thompson was not present (PN-1868).*





# chromatography goes continuous



PN-1869



Rosemary Nicholas, Fox's assistant, removes separated meat pigments from column. They are seen as dark bands—the slower-moving myoglobin is above the hemoglobin (PN-1870).

CHROMATOGRAPHY, until now basically a research tool, may soon have exciting applications in industry. The breakthrough: A practical device for performing the process continuously.

Chromatography is a method for separating a substance into its constituents. In column chromatography, for instance, a mixture of substances passes through a column of material so that each component of the mixture, traveling at its own rate, emerges separately. Modern chromatography is a highly automated and sophisticated technique, one just as important to industrial processing as to research. Yet it is essentially a batch process, not adaptable to the continuous operation demanded by modern high-speed technology.

Theoretically, a way to make chromatography continuous has been known for 20 years. The principle can be explained by envisioning several rowers crossing a swiftly flowing river. The slow rowers are carried downstream farther than the fast row-

ers, so they all reach the shore at different points. Now if the usual chromatographic column were flattened out and joined at the ends to make a hollow cylinder that revolves endlessly, a sample applied at the top of the cylinder would separate into its components, each making its way to the bottom. But before the components emerged at the bottom, the revolving cylinder would carry the slow-moving components farther around the circle than the fast-moving ones.

Using this principle, ARS chemist J. B. Fox of the Eastern utilization research laboratory in Philadelphia, Pa., constructed a laboratory-scale continuous chromatography apparatus. In his apparatus, the space between two concentric plastic cylinders is filled with gel particles. This gel cylinder is rotated by a turntable past a hollow needle through which the sample is applied to the top of the gel. A buffer solution constantly flowing through the gel carries the sample components to the bottom.

For this system to work, flow

through the gel must be uniform and the top surface of the gel must be absolutely flat. Present-day molecular sieve gels have the necessary uniformity to meet the first requirement. The second is achieved by a tiny "plow" that is lowered to the top of the gel to level the surface as the cylinders rotate.

Fox is now using his apparatus routinely in meat research to separate and purify the pigments myoglobin and hemoglobin. Since the device is easy to operate, has few moving parts, and does not need to be attended constantly, Fox envisions widely diversified industrial applications. In the enrichment of skim milk, for example, he has demonstrated that the first step, separating the proteins from the lactose and salts, can be performed continuously with his apparatus. Adopting the present laboratory device to industrial scale may also permit continuous chromatography to be applied to preparing food products, making pharmaceuticals, and recovering usable products from waste materials. ■



# Natural curbs limit plant intake of ARSENIC RESIDUES

**T**WO NATURAL soil phenomena—adsorption and leaching—keep arsenic residues from accumulating excessively in plants.

But with repeated applications of arsenical pesticides, residues do accumulate in the soil. As part of research directed toward reducing the potential hazards of these accumulations, the long-term residual effects of arsenical pesticides were studied by ARS chemist E. A. Woolson and biochemist P. C. Kearney at Beltsville, Md., and soil chemist J. H. Axley of the Maryland Agricultural Experiment Station, College Park.

They tested 70 soil samples taken from U.S. areas known to have received repeated and frequently heavy treatments of lead arsenate insecticide. These samples contained 10 to 2,500 parts per million arsenic. While 5 to 15 ppm arsenic occur naturally in most soils and some have up to 40

ppm, the high concentrations in this study resulted from previous heavy applications to control insects in orchards.

Arsenic residues are not generally harmful to plants because iron and aluminum cations in the soil chemically adsorb (tightly bind) and thus detoxify the inorganic arsenate. When these cations are plentiful, arsenic accumulates as relatively insoluble salts that are not absorbed by plant roots; when scarce, plants usually die or are stunted before significant residues can accumulate in them.

Leaching also reduces root uptake of arsenic residues. When the supply of iron and aluminum is low, arsenic moves downward through the soil and away from the plant root area. For example, in samples of a sandy Florida soil called Lakeland, taken at 1- to 6-foot depth, the researchers found concentrations of arsenic amounting

to 48 pounds an acre. However, the 12-inch surface layer contained a concentration equal to only 34 pounds an acre.

Finer-textured soils with a high clay content accumulated more arsenic even though they contained slightly less iron and aluminum. Forty-seven percent of the arsenic applied to fine-textured New York soils remained in the surface 6 inches while about 3 percent was found in the same depth of Lakeland soil.

In pot studies, 250 ppm arsenic in soil reduced corn growth by some 50 percent when it was harvested at 4 weeks. Under these conditions, the dry plant material contained 10 ppm arsenic.

Researchers at Beltsville are now studying the possibilities of removing arsenic from the root zone by leaching or by the use of iron and aluminum soil additives to make the arsenic less available to plants.

Research also is being conducted on the behavior of organic arsenicals and on their ultimate fate in soil. ■



*Above: Woolson takes samples of soil containing sodium arsenate (0170A27-7). Top right: Four solutions are added to dried samples to extract four forms of arsenic (0170A28-5). Bottom right: As extracts are distilled, Juanita Yates traps arsenic gases in solutions. When reagents are added, arsenic will turn blue—the darker the blue, the more arsenic (0170A27-10).*





**S**OIL MICRO-ORGANISMS—sort of the piranha of the profile—are being used to break down nitrates in irrigation drainage water.

The process, denitrification, may mean a whole new ballgame in cleaning up irrigation drainage and lowering the odds on cutting nitrate pollution in some of the nation's lakes and streams from that source.

Nitrate, a principal ingredient of many chemical fertilizers, is sometimes carried away by field drainage systems and discharged into public waters, where (along with phosphorus from sewage treatment plants) it promotes algal growth.

Breaking down nitrates by denitrification is not new—ARS is using it to clean up secondary sewage effluent from the Phoenix, Ariz., sewage treatment plant (AGR. RES., Dec. 1969, p. 8)—but for the first time it's being used in a different way in irrigated fields of the San Joaquin Valley near Firebaugh, Calif.

Denitrification occurs in soil where there is (1) lack of free oxygen (anaerobic condition), (2) food for bacteria (crop residues), and (3) nitrate (from fertilizer and natural sources). Under anaerobic conditions, free oxygen is lacking for bacterial metabolism so microbes break down nitrate ( $\text{NO}_3$ ) using part of the oxygen in the molecule for respiration. The part of the molecule that's left disperses as nitrogen gas and gaseous oxides of nitrogen.

Field studies of denitrification stemmed from successful laboratory research by ARS soil scientists B. D. Meek and L. B. Grass, agricultural engineer L. S. Willardson, and chemist A. J. MacKenzie stationed at Brawley, Calif.

If this approach works as well in the field as it has in the laboratory, it may mean a cleaner San Francisco Bay, since the San Joaquin Valley drains into the Bay. And, if the Firebaugh studies are successful, there is



*At an observation well, ARS research technician M. J. Huber installs two nylon tubes to sample the air and the drainage in a tile line (PN-1846).*

## NITRATE BREAKDOWN

little reason to doubt that the idea can work in other parts of the country as well.

In creating anaerobic conditions under irrigated fields, drain tiles must be deeper than normal. At Firebaugh, tiles have been placed 6,  $7\frac{1}{2}$ , and 9 feet deep in order to have them below the water table where these conditions exist. The three levels will allow scientists to determine the best depth for adequate denitrification. In actual practice, only one depth will be used.

The top of the water table will be kept at a predetermined level by draining the field from risers at the end of the drainage line. The risers will have the same effect as an overflow pipe in a swimming pool or sink, and the water in the field will drain off at the height of the risers.

In the laboratory, the scientists say, nitrate disappearance was rapid. In one test in soil columns drained at the 6-foot level, nitrate concentrations of 16 parts per million almost disappeared within a week. Water containing more than 45 ppm nitrate may

be unsafe for human consumption.

Two serendipital effects may be noted in deep tiles—phosphorous clings tenaciously to soil particles and probably will not drain off through the deep tiles and, more important to irrigators, tiles will not clog up with manganese and iron oxides. The latter problem occurs when aerobic conditions are present and the oxides coat the inside of drain tiles exposed to the air. That involves other bacterial activity and is what the Brawley scientists started out to solve when they branched out to include the denitrification process.

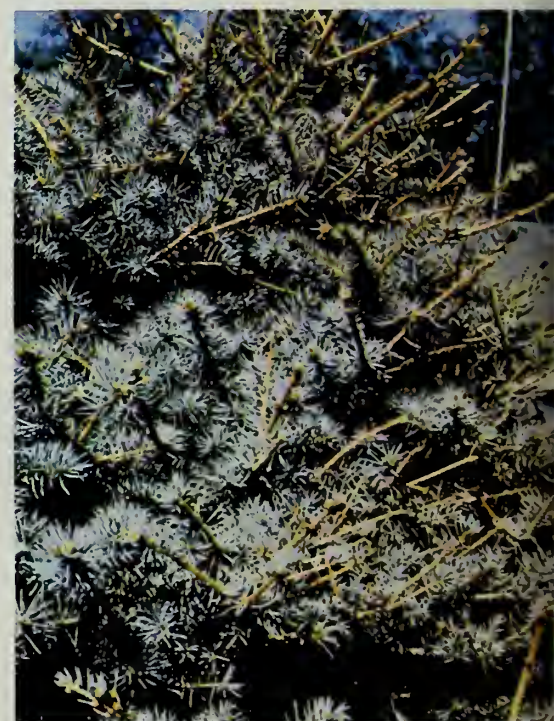
Every possible reading will be made at the Firebaugh site within the next couple of years, including soil samples, water samples, temperature readings, electrical conductivity studies, and of course, nitrate level readings both in the water table and in the drain water.

ARS, the Bureau of Reclamation, and the Soil Conservation Service are cooperating in the Firebaugh field studies. ■





**Top right:** Typical sulfur dioxide injury to white birch leaf at left followed 2 hours of exposure to 2 ppm (color: A.P.Dam-0-4; b&w: BN-35293). **Bottom right:** White Cascade petunia leaves show injury from ozone. White-flowered varieties tend to be more susceptible than those with colored flowers (color: A.P.Dam-0-1; b&w: BN-35289). **Far right:** In an air pollution laboratory jointly maintained by ARS and the U.S. Department of Health, Education, and Welfare's Public Health Service, science aide Eileen Murphy weighs soybean plants exposed to ozone and sulfur dioxide mixtures at levels often measured around Eastern cities. PHS plant physiologist David Tingey measures soybean plants to see if height was reduced (A.P.Res-0-9; b&w: BN-35288).



**Cover:** Several species of grass are exposed to a mixture of ozone and sulfur dioxide metered into the airstream circulating in a plant growth chamber (b&w: 1269A235-5). **Above:** Heggstad compares two Norland potato plants of the same age. Plant at right was grown in the lab in nonfiltered air; the other was grown in a greenhouse section provided with carbon-filtered air (color: A.P.Res-0-7; b&w: BN-35287). **Top right:** Fluoride injury to blue spruce causes loss of young needles. Fluorides, emitted during processing of phosphate fertilizers, aluminum, steel, brick, and pottery, produce a characteristic injury in plants, but plant sensitivity varies greatly. Some species may accumulate more than 500 parts per million fluoride in a single season without visible symptoms; others are injured if they accumulate as little as 50 ppm (color: A.P.Dam-0-3; b&w: BN-35290). **Bottom right:** Sulfur dioxide injury to bromegrass tends to develop as streaks because veins of grass species are parallel (color: A.P.Dam-0-2; b&w: BN-35292).





Our shadowed skies put

# **PLANTS UNDER SIEGE**

**A**IR POLLUTION INJURY to vegetation is increasing across the United States, causing losses estimated at more than half a billion dollars annually . . . and rising.

Atmospheric contamination took a heavy agricultural toll in parts of Southern California after World War II, forcing some truck crop, citrus and orchid growers out of business. The growth of vegetables, tobacco, and certain ornamentals is now threatened in the East.

To combat this problem, ARS is conducting research at its modern Plant Air Pollution Laboratory in Beltsville, Md., (AGR. RES., Jan. 1967, p. 3) and at several field stations. The research is cooperative with the National



Air Pollution Control Administration of the U.S. Department of Health, Education, and Welfare.

At the Beltsville laboratory, plant pathologist H. E. Heggstad, plant physiologist C. W. Chang, and plant pathologist R. K. Howell are studying the nature of pollutant-caused injury and are identifying species resistant to pollutants—particularly photochemical oxidants—that affect plant growth.

Photochemical oxidants are toxic chemicals such as ozone and peroxyacetyl nitrate (PAN) formed by the action of sunlight on nitric oxide, nitrogen dioxide, and certain reactive hydrocarbons (e.g. unburned gasoline) produced during fuel combustion.

In 1966, ARS plant physiologist H. A. Menser and Heggstad discovered that ozone and sulfur dioxide in combination more seriously affected plants than either did alone. Their finding upset the long-held notion that the two chemicals counteract the toxicity of one another. (AGR. RES., Oct. 1966, p. 3).

The Plant Air Pollution Laboratory is equipped with special fumigation chambers for growing and exposing plants to various types and amounts of pollutants, allowing scientists to observe the effects of the chemicals on the plants. In larger-scale studies, greenhouses specially equipped with activated carbon filters enable the researchers to compare plants grown in almost pollution-free environments with those grown under normal greenhouse conditions in unfiltered air.

The research shows that most of the plant damage is due to suppression of growth—as much as 50 percent in some crops. Some growth inhibition may occur without visible injury. Moreover, the most susceptible plants are prematurely aged and weakened by polluted air. These plants often are susceptible to further damage from insects, diseases, and nematodes.

Until air pollution is significantly reduced, about the only way to control crop losses in the field is through identifying and developing resistant plants. Susceptibility or resistance to air pollution, however, usually varies within plant species; thus, not only each species but each variety within the species must be tested for susceptibility or resistance to a wide range of pollutants.

In experiments thus far, Heggstad and his team note that some plants fared almost as well in unfiltered air as in filtered air—juniper, arborvitae, Acala SJ-1 cotton, American and English holly, pyracantha, and tomato, for example. By contrast, other plants including sycamore, boxelder, petunia, potato, certain forage grasses, tobacco, radish, pinto bean, and spinach grown in filtered air

proved healthier and higher yielding. Yields of Bel W-3 tobacco as well as Norland and Irish Cobbler potatoes grown in filtered air were almost 100 percent higher in summer months.

In future research, some of the plants already tested will be grown again during a different time of year to determine if season of growth is significant. And the experiments also will involve other major crops.

Once scientists establish the reaction of major crop and ornamental species and varieties to particular pollutants, growers will be able to select crops best suited to withstand prevailing local environmental conditions. And looking toward the future, this research will aid plant breeders in developing plants that can maintain acceptable yields until the air pollution problem is abated. ■



*Activated charcoal is the heart of the Beltsville laboratory's filtration system. One pound of charcoal from a carbon filter has a surface area equal to about 200 acres, and each filter contains 45 pounds. Each 10,000-cubic-foot greenhouse section is equipped with 12 filters. Air in these sections can be completely changed every minute (1069A89-5).*



# FORECASTING CROP YIELDS

**R**EMOTE SENSING TECHNIQUES for estimating crop yields have advanced another step.

Preliminary studies in the Lower Rio Grande Valley of Texas show that film density differences in aerial color infrared photographs are related to variations in certain "yield indicators"—such as number and height of plants—measured on the ground.

Once techniques are fully developed, remote sensors in aircraft or satellites may provide rapid collection of data needed by USDA's Statistical Reporting Service in estimating crop yields. Among others, growers, shippers, transporters, and processors use yield estimates in planning orderly marketing of farm produce.

SRS mathematical statistician D. H. Von Steen, ARS soil scientist R. W. Leamer, and ARS research technician A. H. Gerbermann collected remotely sensed and ground data on cotton, grain sorghum, carrots, cabbage, and onions. They selected farmers' fields to provide variations in maturity, yield potential, plant populations, and soils.

The scientists made field counts or measurements on four or five yield indicators for each crop at monthly intervals. They also determined film density by scanning the transparencies with an isodensitracer, using appropriate filters in the instrument to measure density of the red, blue, and green layers of film. Average density readings were then related to plant counts and measurements obtained from the sample plots.

The researchers obtained statistically significant correlations between yield indicators and film density for all crops except grain sorghum, for

which only 1 month's data were obtained. Von Steen says acceptable yield estimates should be possible with data obtained from aerial color infrared photographs if some ground information is obtained at the time of each overflight.

When all of the data were considered, the following yield indicators correlated with film density:

**Onions:** Number of plants per acre, percentage vegetative cover, plant height, bulb size (each with three filters—red, green, and blue—and without a filter).

**Cabbage:** Number of plants, per-

centage vegetative cover, plant height, weight of harvested cabbage per plot, size of head (two filters each).

**Carrots:** Size of crown (three filters and without filter), plant height (two filters), number of plants (one filter).

**Cotton:** Number of open bolls per acre (three filters), number of open and partially open bolls per acre (two filters).

The Texas Agricultural Experiment Station and the National Aeronautics and Space Administration cooperated in the research. ■

*In remote sensing studies, science technician Clarita Coulson operates a microdensitometer which measures the variations in film emulsion densities of selected areas after scanning by the isodensitracer (ST-4705-15).*





Laboratory technician R. E. Minor injects guinea pig with challenge dose of bacterial spores at edge of large rump muscle—the place Macheak found best for challenge work (PN-1871).



## Improving potency tests of Blackleg Bacterin

IMPROVED potency evaluations for blackleg bacterin are providing cattle with surer protection against blackleg infections.

Blackleg bacterin, made from killed *Clostridium chauvoei* bacteria, has traditionally been evaluated on guinea pigs, not cattle. Aside from the expense, it was impossible under laboratory conditions to infect cattle consistently with bacteria to challenge the bacterin's effectiveness. Even guinea pigs, which are more susceptible to blackleg than cattle, sometimes failed to become infected in laboratory tests because *Cl. chauvoei* bacteria reproduce only under anaerobic conditions—that is, in places where air is excluded. They can survive in soil for years in spore form, but they infect

and multiply only when they get into the oxygen-short dead tissues of wounded cattle.

The problem was solved by ARS regulatory veterinarian M. E. Macheak, who directs quality control tests on bacterial products at the National Animal Disease Laboratory, Ames, Iowa. A central feature of his complex technique is a good challenge injection site at the edge of the large rump muscle. The revised technique all but eliminates unsuccessful tests due to unsuccessful challenge infections. In addition, it has proved a valuable aid in studies to clarify the accuracy of the traditional guinea pig test in indicating a bacterin's ability to protect cattle.

In the studies, Macheak vaccinated

a group of calves with a bacterin rated satisfactory by guinea pig tests. After the vaccination had a chance to take effect, he challenged the calves with 10 milliliters of a 1:1000 dilution of bacterial spores. All calves survived. But a second group of calves vaccinated with a bacterin rated unacceptable suffered 100 percent mortality when faced with the same challenge. A third group of 18 calves not vaccinated against blackleg suffered only 66 percent mortality, and the casualties occurred much later.

The critical phase of the studies sought to show whether the traditional pass-fail score for blackleg bacterin tests—protection of seven out of 10 guinea pigs—was really a meaningful cutoff point.

Four marginal samples of commercial blackleg bacterin that in 12 tests had protected an average of five out of 10 guinea pigs, were compared with a very low potency sample that had protected an average of only two out of 10 guinea pigs.

When the marginal products were tested in cattle, 31 percent of the calves died after challenge. With the low-potency product, 40 percent of the calves died.

In addition, Macheak tried a correlation on cattle with an ARS test bacterin that, over many years of guinea pig tests, had protected about half of the laboratory animals from challenge. Results showed that this bacterin protected seven out of 15 cattle on trial. Macheak says that these correlations are about as good as is possible to attain.

Based on these findings, ARS biometricians have worked out a statistic interpretation of guinea pig potency tests in terms of protection for cattle. The scientists can show how many guinea pigs should be tested—and in what sequence—to obtain at least 95 percent surety that a bacterin passing the guinea pig test will protect at least 98 percent of the cattle vaccinated. ■



# KETOSIS... solving a two-part puzzle

**T**HE CAUSES of pathogenic and non-pathogenic forms of ketosis, a metabolic disorder of animals under nutritional stress, have now been clarified, opening the way for study of the pathogenic form.

In sheep, pathogenic ketosis strikes while pregnant ewes are carrying more than one fetus. In cattle, it occurs shortly after the onset of lactation in cows that are high producers of low-fat milk. Also known as pregnancy disease of sheep, pregnancy toxemia, acetonemia, and ketonemia, this form of the disorder nearly always is fatal.

An elevated level of organic compounds called ketones in the blood and urine is always associated with ketosis. But in the nonpathogenic form, for reasons previously not understood, a high level occurs without visible symptoms. This form is seen only in ruminants.

J. H. Adler, veterinary physiologist at Hebrew University-Hadassah Medical School, Jerusalem, confirmed earlier studies in England indicating the reasons for elevated ketone levels: In pathogenic cases, excess ketones result from metabolism of body fats; in nonpathogenic cases, excess ketones are end products of microbial fermentation in the rumen.

Adler, whose research was supported by a Public Law 480 grant from ARS, says that pathogenic ketosis should be regarded as glucose starvation. While sheep with ketosis usually are thin, the disorder can occur in fat ewes whose energy supply in terms of calories is adequate if they are deficient in glucose.

In the absence of adequate glucose, two-carbon fat fragments condense to form four-carbon compounds, which are the basis for ketone production. With adequate glucose, the two-car-

bon fat end products oxidize to carbon dioxide and water.

The Israeli scientist says two characteristics of domestic sheep and dairy cattle predispose them to ketosis.

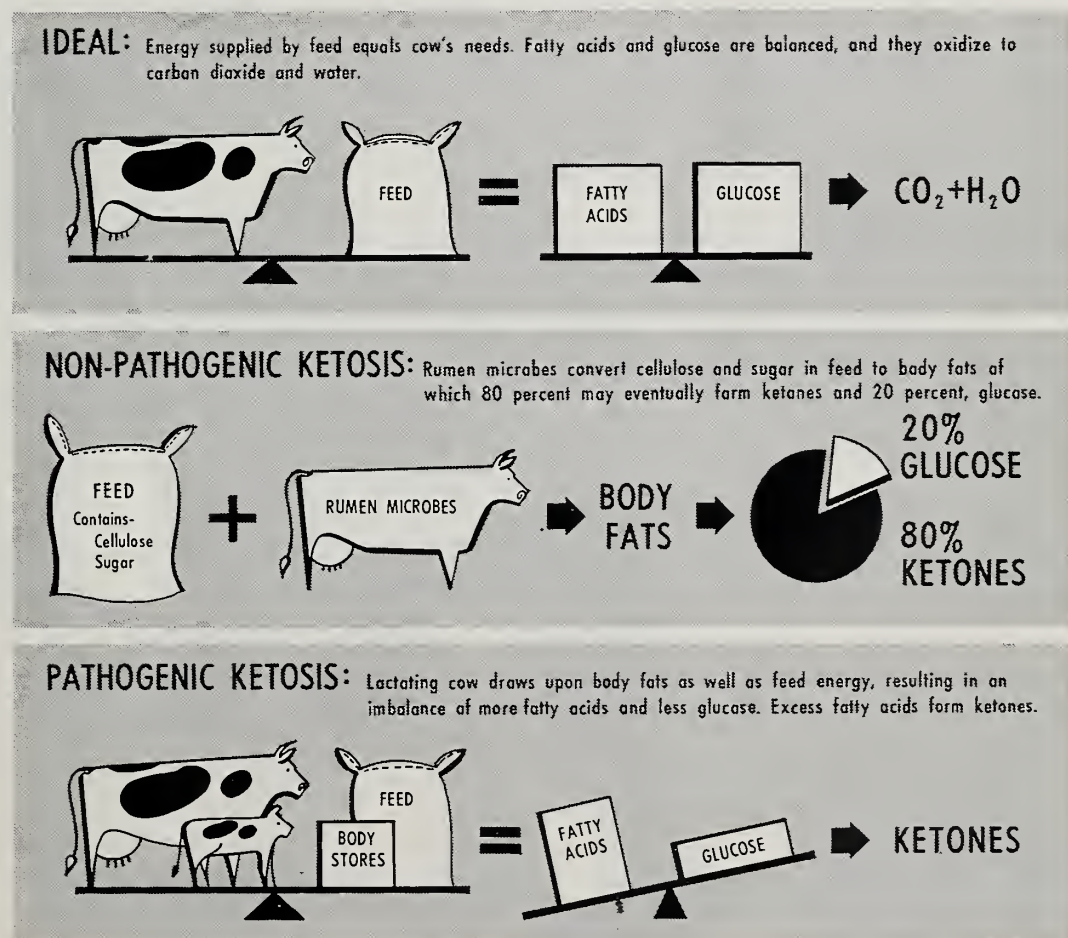
Both have been selected to produce beyond their natural needs for man's benefit—the ewe to deliver twins or triplets and the cow to give more milk than her calf requires. Reproduction, with its high glucose requirements, selectively drains glucose away from the pool of calories available for maintenance. Ketosis accordingly occurs at the time of extra stress—pregnancy in the ewe and lactation in the cow. And the cow producing high-fat milk uses more of the fat from the calorie pool in milk production and consequently is less susceptible to ketosis than the cow that gives low-fat milk.

In addition, the ruminant is constantly on a relatively high ketogenic diet because of its digestive mechanism. Rumen micro-organisms convert most of the dietary sugar and all of the cellulose to fatty acids. Eighty percent of the fatty acids are ketogenic, and 20 percent are propionic acid, which may be converted to glucose.

Diet during pregnancy or lactation can enhance or reduce the chances of pathogenic ketosis, Adler points out.

If the diet consists entirely of roughages, fermentation in the rumen favors production of acetic acid, which cannot be converted to glucose but can form ketones. But if the diet contains a high ratio of grain and concentrates, rumen fermentation favors production of propionic acid, which can be converted to glucose. ■

PN-1872





**R**ICE IS ONE STEP CLOSER to becoming a practical source for enriching protein-deficient foods. Japanese scientists have isolated and stabilized a high-protein concentrate from rice endosperm cells, which store food for the life support system of the seed embryo.

The existence of protein bodies in the peripheral, or aleurone, layer of rice endosperm has been known for many years, but virtually nothing was known about the sites of other protein accumulation within the endosperm. Under a Public Law 480 grant from ARS, Japanese scientists isolated these protein bodies from rice endo-

## protein supplement ... from rice?

sperm and found that they contained 60 percent protein; remaining constituents were described as lipid, carbohydrate, phytic acid, RNA, thiamine, and niacin.

The researchers also noted that the protein bodies were fragile; they tended to break down in fluids and at temperatures higher than 4° C. Preliminary tests showed that the protein bodies were fairly stable in a neutral pH of about 7 if stored at 0° C. for a short period, but broke down easily or aggregated together as the pH was increased or decreased.

After further tests, the scientists concluded that the best conditions for stability were those that included a dilute phosphate buffer to resist changes in neutral pH and a dilute suspension medium of sucrose at 0° C.

In describing electron micrographs of the protein bodies, the Japanese

were the first to report an unusual characteristic in the protein subcellular structure. The micrographs revealed thick and thin concentric layers of electron-dense material similar in appearance to the annual rings of a tree. The significance of this structural pattern is not yet known.

The Japanese groundwork points to the desirability of identifying the proteins within the protein bodies and of characterizing their enzymes. To do so would be a major step toward utilizing rice protein in new and improved foods.

This 4-year project was conducted under the direction of principal investigator H. Mitsuda at the University of Kyoto, Kyoto. ARS biochemists R. L. Ory and A. J. St. Angelo, the sponsoring scientists, are stationed at the ARS Southern utilization research laboratory, New Orleans, La. ■





## Continuous Process for Celery Oil

The extraction of celery oil from plant parts usually discarded as waste has now been adapted to continuous processing.

ARS chemists C. W. Wilson, III, M. K. Veldhuis, R. E. Berry, and C. J. Wagner, Jr., developed the continuous process at the Fruit and Vegetable Products Laboratory, Winter Haven, Fla. ARS scientists had previously extracted oil from wastes through a batch process (AGR. RES., Feb. 1967, p. 13).

To obtain the oil, celery leaves, tops, and outer ribs are heated to drive off 10 to 20 percent of the moisture which carries with it the volatile celery oil. The vapors are trapped and the oil is separated from the moisture.

Celery is a highly desirable flavoring in processed foods and, because fresh celery is not always available, processors are using increasing amounts of dehydrated celery. But the heat used in removing moisture from celery also drives off some of its flavor and aroma, which celery oil can restore. Oil from waste material, however, is more flavorful and potent than oil extracted from celery seed or made synthetically.

Although only about 5 ounces of oil is obtained from a ton of raw material treated by the new process, these 5 ounces are theoretically enough to flavor a ton of dehydrated celery. The total material grown per acre in a celery crop is on the order of 30 to 45 tons. About 10 to 15 tons, which now are left in the field, could be processed for oil by this method.



*Aerial view looking east-southeast shows the 14-to-2-row soybean-corn planting pattern. At far right is the control area (PN-1873).*

## Corn Windbreaks

Soybeans, sheltered by two-row corn windbreaks, have shown yield increases of as much as 6 bushels per acre in a western Minnesota study.

The sheltered plants also grew taller, produced more dry matter, and had larger and more leaves than plants in unsheltered control plots.

The Minnesota area, like some other parts of the country, lends itself to this research because of its prevailing winds. Near Morris, soybeans and corn were planted at the same time in rows nearly perpendicular to south-to-southwest prevailing winds. Seven seed hoppers in an 8-row planter were filled with soybeans and the eighth with corn. The planting pattern was 14 rows of soybeans to two of corn.

ARS soil scientist J. K. Radke knows that the increased yields were brought on indirectly by changes in wind movement and intensity. But most likely direct cause, he says, is

variation in how the water was used. Although there was no *statistical* difference in water use, more water went through the plants in the sheltered areas than in the control plots.

Other possibilities include light stress—wind blowing leaves so that the reflective underside intercepts light—and undetected physical damage caused by the wind.

Studies continue to find the exact cause in hopes of applying the knowledge to other situations.

## Hormone Blocks Beetle Growth

Synthetic insect hormones may be useful as a new kind of insecticide to help control the Mexican bean beetle.

Nine experimental synthetic hormones prevented normal adult development when applied to prepupae or young pupae in laboratory tests. As little as 10 nanograms—10 billionths of a gram—of some compounds was effective. ARS entomologists W. F. Walker and W. S. Bowers conducted



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the tests at Beltsville, Md. The treatments also prevented egg hatch when egg masses were briefly dipped into a liquid formulation of hormone diluted as low as 1 part per million. The vapor from very small quantities of these hormones was effective when prepupae or eggs were sealed in treated petri dishes.

Adult beetles that fed on hormone-treated leaves laid eggs that failed to hatch, and larvae that fed on the leaves developed into abnormal pupae.

Eight of the synthetic hormones are terpenoid ethers. The ninth was methyl *trans, trans*, 10,11 epoxy farnesenate, which was also effective at higher doses than were used with the ethers.

## New Light on Grass Evolution

Indian researchers have found a new low chromosome number in the grass family, a finding that may furnish plant geneticists with a better understanding of grass evolution.

Among the approximately 10,000 species of grass, chromosome sets have been found to range from four to nearly 200. The primary numbers of five and seven chromosome sets have been considered the basic number of the grass family.

In the current ARS-sponsored Public Law 480 work, directed by Rui D'Cruz at the College of Agriculture, Poona, one species, *Iseilema anthe-phoroides* Hack., was found to have only three sets of chromosomes. Available evidence suggests that species

with this low chromosome number may have evolved from species having higher numbers.

The scientists note that by crossing species having a low number of chromosome sets with species having higher numbers, geneticists may learn more about the biological makeup of complex parental species.

ARS scientists say it is doubtful that *I. anthe-phoroides* will be adapted in the United States. However, this spin-off is part of a 5-year genetic study begun in 1965 on forage grasses belonging to a large and complex tribe, the *Andropogoneae*. Purpose of the work is to find and improve promising species for use in revegetating range lands in India and the United States.

Sponsoring scientists for this project are agronomist A. W. Hovin, formerly of ARS and now with the University of Minnesota, Minneapolis, and ARS research geneticist J. B. Powell, Tifton, Ga.

## Halogeton May Cause Sick Cattle

Cattle that become sick after grazing winter ranges in the West may be suffering from sublethal halogeton poisoning.

Primarily a disease of sheep, halogeton poisoning occasionally affects cattle. Poisoning is caused by eating *Halogeton glomeratus*, a plant that grows wild in the Western States. Livestock do not ordinarily consume toxic amounts unless they are hungry.

ARS toxicologist L. F. James,

Logan, Utah, recently investigated eight similar cases of suspected sublethal poisoning occurring in western Box Elder County, Utah. In each case, the cattle had been grazing desert ranges infested with halogeton and were driven along roads lined with it.

The animals became stiff after being driven about 5 miles and had difficulty moving. Stiffness appeared first in the front legs and then progressed until all limbs were involved. Some cows lay down and had to be trucked to where they could be hand-fed and watered. Others became weak, but were left to themselves and recovered. Deaths occurred infrequently.

Suspected sublethal halogeton poisoning of cattle had not occurred until halogeton began growing in the area, and livestock handled similarly in halogeton-free areas had no symptoms of poisoning.

Stockmen found that when cattle were fed hay before being driven along halogeton-infested areas, they did not develop signs of the poisoning.

**CAUTION:** In using pesticides discussed in this publication, follow directions and heed precautions on pesticide labels. Be particularly



careful where there is danger to wildlife or possible contamination of water supplies.